19. W. L. Crepet, K. C. Nixon, in *The Anther, Form, Function and Phylogeny*, W. G. D'Arcy, R. C. Keating, Eds. (Cambridge Univ. Press, New York, 1996), pp. 25–57.
20. E. M. Friis, K. R. Pedersen, P. R. Crane, *Int. J. Plant Sci.* **161** (suppl. 6), S169 (2000).
21. K. C. Nixon, W. L. Crepet, D. A. Stevenson, E. M. Friis, *Ann. Mo. Bot. Gard.* **81**, 484 (1994).
22. M. J. Donoghue, J. A. Doyle, in *The Hierarchy of Life,* B. Fernholm, K. Bremer, H. Jornvall, Eds. (Elsevier, New York, 1989), pp. 181–193.
23. H. Loconte, D. W. Stevenson, *Brittonia* **42**, 197 (1990).
24. _____, *Cladistics* **7**, 267 (1991).
25. J. A. Doyle, M. J. Donoghue, *Bot. Rev.* **52**, 321 (1986).
26. P. K. Endress, *Diversity and Evolutionary Biology of Tropical Flowers* (Cambridge Univ. Press, Cambridge, 1994).
27. W. Hagemann, in *Contemporary Problems in Plant Anatomy,* R. A. White, W. C. Dickinson, Eds. (Academic Press, Orlando, FL, 1984), pp. 301–350.
28. P. Leins, C. Erbar, *Bot. Acta* **104**, 336 (1991).
29. J. V. Ward, J. A. Doyle, C. L. Hotton, *Pollen Spores* **31**, 113 (1989).
30. T. M. Harris, *The Yorkshire Jurassic Flora. II. Caytoniales, Cycadales & Pteridosperms* (British Museum [Natural History], Oxford, 1964).
31. G. Sun, S. L. Zheng, D. L. Dilcher, Y. D. Wang, S. W. Mei, *Early Angiosperms and Their Associated Plants from Western Liaoning, China* (Shanghai Scientific and Technological Education Publishing House, Shanghai, 2001).
32. E. A. N. Arber, J. Parkin, *Ann. Bot. (London)* **22**, 489 (1908).
33. R. R. von Wettstein, *Handbuch der Systematischen Botanik* (Franz Deuticke, Leipzig, ed. 2, 1907).
34. V. A. Krassilov, *Angiosperm Origins: Morphological and Ecological Aspects* (Pensoft, Sofia, Bulgaria, 1997).
35. P. R. Crane, *Ann. Mo. Bot. Gard.* **72**, 716 (1985).
36. T. Delevoryas, *Palaeontographica Abt. B* **121**, 122 (1968).
37. W. L. Crepet, *Palaeontographica Abt. B* **148**, 144 (1974).
38. M. P. Doludenko, E. R. Orlovskaya, *Jurassic Flora of the Karatau* (Science Press, Moscow, 1976).
39. S. E. Wang *et al*., *Jurassic System. In Stratigraphy of China* (Geological Press, Beijing, 2000).
40. Q. Ji *et al*., *Prof. Pap. Stratigr. Palaeont.* **27**, 74 (1999).
41. S. A. Ji, Q. Ji, K. Padian, *Nature* **398**, 573 (1999).
42. J. F. Zhang, *Paleoworld* **11**, 150 (1999).
43. C. H. Lo *et al*., *Geochemica* **28**, 406 (1999).
44. D. F. Wang, *Bull. Chin. Acad. Sci.* **7**, 65 (1983).
45. P. E. Smith *et al*., *Can J. Earth Sci.* **32**, 1426 (1995).
46. K. C. Nixon, *Cladistics* **15**, 407 (1999).
47. Consensus cladogram of most parsimonious trees for analysis of 173 living taxa of seed plants, plus the fossil *Archaefructus*. Various analyses included 1628 molecular characters and 17 to 108 morphological characters. The molecular characters are based on the three-gene matrix (*rbcL*, *atpB*, *18s*) that was recently published for 567 species (*2*). Taxa were selected to provide a good representation of variation throughout the angiosperms, including a dense sampling of the so-called basal angiosperms. The tree shown was generated with a matrix of 1645 characters (17 morphological characters, including only those relevant characters that could be scored for the fossil). Parsimony analysis was undertaken using the parsimony ratchet of Nixon (*46*), with numerous runs of 200 replications for each analysis. In all analyses, *Archaefructus* is a sister taxon to the angiosperms as shown in this tree. Depending on the data set used, the overall length of the tree varied, with an overall consistency index of ~0.18 (consistent with the original three-gene analysis). The taxa *Cycas*, *Bowenia*, *Zamia*, *Ginkgo*, *Ephedra*, and *Pinus* represent the modern gymnosperms; the other taxa in the analysis are angiosperms (flowering plants). Note that this data set does not address the question of whether the gymnosperms are monophyletic, because no taxa outside of the seed plants were included. The tree has been drawn to be neutral on this point, and it supports either hypothesis equally. The tree differs from the original three-gene analysis only in the position of *Ephedra*, which in these trees is more consistent with analyses of other genes that place gnetopsids with Pinaceae, suggesting that the morphology may play a positive role in resolving discrepancies.
48. See supplemental material on *Science* Online (www.sciencemag.org/cgi/content/full/296/5569/899/DC1).
49. Supported by the National Natural Science Foundation of China (projects 39970050 and 39770059), Key Project of Ministry of Land and Resources, China, Special Project Stz. 98-1-02 (Chinese Academy of Sciences), and by NSF grants INT-0074295 and DEB 0090283 and the Becker/Dilcher Endowment for Paleobotany of the University of Florida Foundation. We thank T. A. Lott for assistance with photography, illustration, and manuscript preparation; K. Simons for diagramming the reconstruction of *Archaefructus*; and J. Y. Liu and C. L. Gao of the Natural Museum of Dalian for their help in observation of some specimens of *Archaefructus*. This paper is University of Florida Contribution to Paleobiology publication number 525.

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Mammal Population Losses and the Extinction Crisis

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The disappearance of populations is a prelude to species extinction. No geographically explicit estimates have been made of current population losses of major indicator taxa. Here we compare historic and present distributions of 173 declining mammal species from six continents. These species have collectively lost over 50% of their historic range area, mostly where human activities are intensive. This implies a serious loss of ecosystem services and goods. It also signals a substantial threat to species diversity.

Population extinctions are a more sensitive indicator of the loss of biological capital than species extinctions. This is because many of the species that have lost a substantial portion of their populations [thus altering ecosystems and perhaps reducing the ability of those systems to deliver services (*1*)] are unlikely to go globally extinct and enter the species extinction statistics in the foreseeable future (*2*). Most analyses of the current loss of biodiversity emphasize species extinctions (*3–5*) and patterns of species decline (*6–8*) and do not convey the true extent of the depletion of humanity's natural capital. To measure that depletion, we need to analyze extinctions of both populations and species. Here we give a rough minimum estimate of the global loss of continental mammal populations. We believe that mammals, because of their great taxonomic diversity and the wide range of ecological niches they exploit, can serve as an indicator of what is occurring in the rest of Earth's biota.

Our data consist of historic (i.e., mostly 19th century) and present-day distributional ranges of all of the terrestrial mammals of Australia and subsets of the terrestrial mammal faunas of Africa, South East Asia, Europe, and North and South America (Table 1 and table S1). These subsets consist of all mammal species whose ranges are known to be shrinking for which we had access to data. They comprise roughly 4% of the ~4650 known species. We assume that loss of range area is due to the extinction of populations, but we do not attempt to equate a given areal loss with a precise number of population extinctions due to the complexities of defining and delimiting populations (*9*). Data were gathered from the specialized literature (Web references). In general, because they are better known, most of our range data are from medium- and large-sized species. Whether globally these are more or less liable to population extinction than medium to small species is a matter of conjecture (*10–12*), but at present there is little reason to assume an important directional bias in our samples. There was no correlation between body mass and range shrinkage in our data ($P > 0.05$, $r^2 = 0.22$). There does remain a possible source of bias in the relative lack of very small species in the total sample (*12*).

The ranges were digitized and the historic and present range areas were calculated. For each species, we estimated both total area occupied historically and percent historic range area now occupied. Using ArcView 3.1, the ranges were superimposed to produce synthetic maps summarizing the losses of species populations in 2 degree by 2 degree quadrats (i.e., the number of species that have disappeared from each quadrat because all of their populations previously located in that quadrat have disappeared). The area of these quadrats, of course, varies with latitude, but the average of such quadrats over land is about 30,000 km^2.

Declining species of mammals in our sample had lost from 3 to 100% of their

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geographic ranges (mean 68 ± SE 2.46), but range lost was above 50% for most (72%) species (Table 1). Species such as Pere David's deer (*Elaphurus davidianus*), which is extinct in the wild, lost 100%, whereas others like Spotted hyena (*Crocuta crocuta*) that have a higher tolerance for human disturbance lost 14%. As expected, there were striking differences between the continents, as shown in Table 1 and Figure 1. The number of populations lost has been greater in areas that are both large and species rich (e.g., Africa and Southeast Asia).

In our analysis, population extinctions today seem to be concentrated either where there are high human population densities, or where other human impacts, such as intensive agriculture, grazing, and hunting, have been severe. Larger mammals are often hunted to extinction or have their habitats preempted (*13*, *14*). The mammal faunal sample from Southeast Asia shows one of the highest losses of species ranges and, thus, of mammal population extinctions: 57% of its quadrats have lost between 75 and 100% of their mammals. In Southeast Asia, human population density is extremely high (e.g., Indonesia, 115 persons per km^2; China, 130 persons/km^2; Pakistan, 190 persons/km^2; India, 305 persons/km^2). Similarly, in North America, the highest percentage losses are in the heavily populated eastern United States.

In Africa, the areas with the highest levels of mammal population extinction do not coincide as well with high human population densities (e.g., Nigeria has 135 persons/km^2), even though there is a positive correlation of human population density with species richness in general (*15*). Rather, the highest percentage of population extinctions have occurred in the region of the Sahara (Mali, 4 persons/km^2; Mauritania, 1.5 persons/km^2), presumably because gazelles and other large herbivores have been hunted to extinction by local people and sport hunters and because of anthropogenic desertification and competition with domestic animals for scarce forage and water (*16*). In recent years, many populations of tropical species such as gorillas (*Gorilla gorilla*) and drills (*Mandrillus leucophaeus*) have been lost in equatorial Africa (e.g., Congo, where there are 20 persons/km^2) (*17*, *18*), but there are no good data on their present geographic ranges. In southern Africa, not surprisingly, the absolute number of extinctions coincides with high population densities of *Homo sapiens*.

Understandably, Australia, which is the continent with the largest number of mammal species extinctions (*12*, *19*), is also a continent showing a widespread severe reduction of populations. Factors causing population and species extinctions there are mainly related to overgrazing, agriculture, forestry practices (including altered fire regimes) (*20*), and, especially, the large numbers of introduced predators and competitors (*21–24*).

In South America, population losses are heaviest in the intensively agricultural southern plains (Pampas region in Argentina),

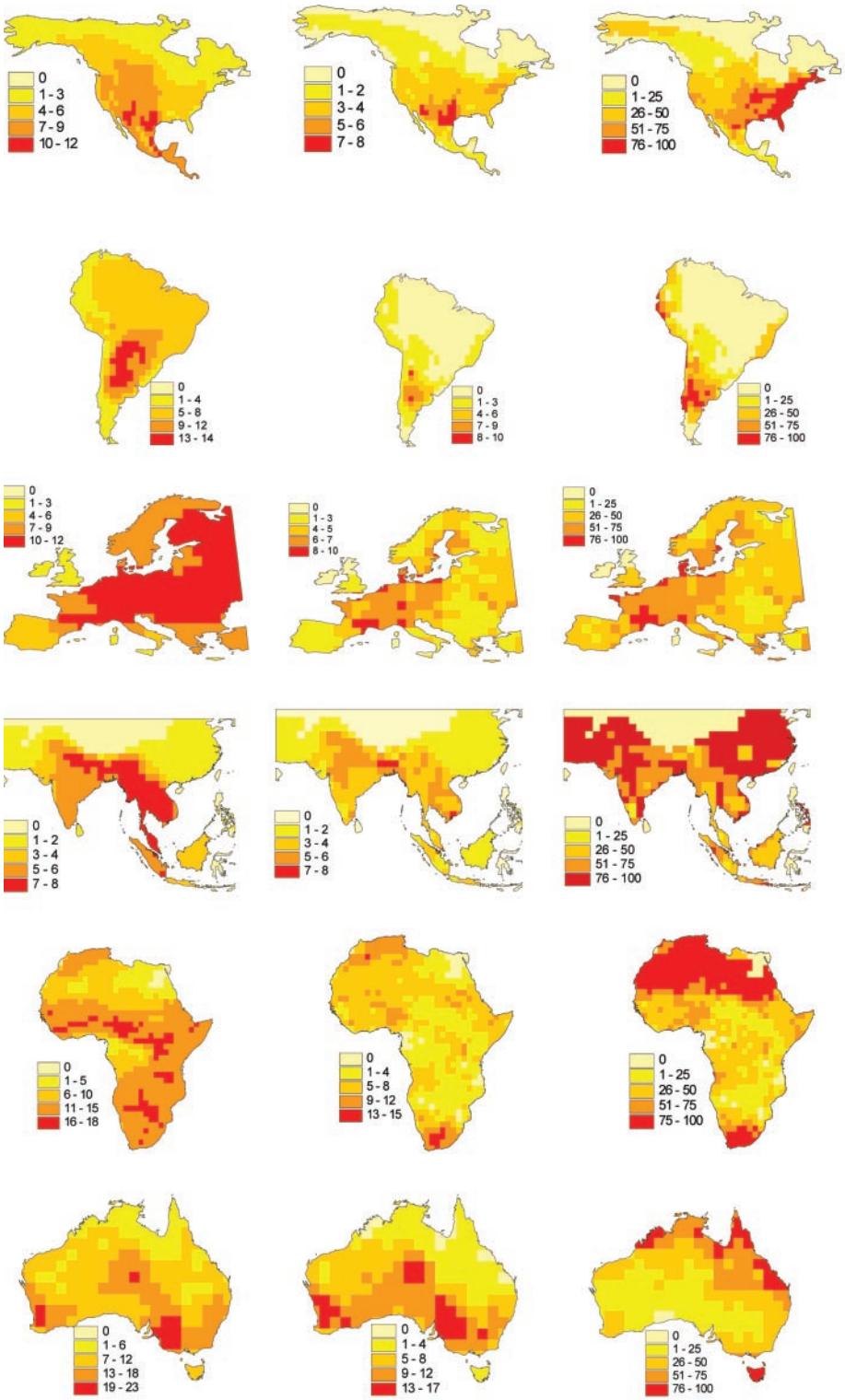

Fig. 1. Historic number of species with populations in each 2 degree by 2 degree quadrat (left column of maps), number of species lost from each quadrat (center column), and percentage of species that have disappeared from each quadrat (that is, percentage of population loss) (right column). All data (top to bottom) from species with shrinking ranges in North America (18 spp.), South America (17 spp.), Europe (15 spp.), Southeast Asia (13 spp.; white quadrats at top, outside of range sampled), Africa (52 spp.), and Australia (58 spp).

Mata Atlantica in Brazil, and coastal Ecuador and Peru. Those areas have been devastated by cattle grazing and unsustainable cropping, and they are among the most degraded of that continent (*25*, *26*). In Europe, no distinct pattern emerges even though the continent has been subject to extensive and severe human alteration. One possible reason is that it is a peripheral region with a depauperate mammal fauna that, by the 19th century, may already have lost most species that would decline in the face of anthropogenic disturbance. For example, the wolf (*Canis lupus*), brown bear (*Ursus arctos*), beaver (*Castor fiber*), and other species had been exterminated in Britain by 1700 (*27*, *28*). Therefore, those species were not included in our historic maps of Britain.

In our sample, declining mammal species have collectively lost over 50% of their continental populations (as judged by area loss). If the proportion of declining species in Australia (22%) is typical of the other continents, this would suggest a loss of more than 10% of all mammal populations. But the Australian proportion of decline may be higher than that of other continents. If we make the conservative assumption that the only declining species globally were those in our sample (4% of the global fauna), a loss of about 2% of all mammal populations would still be suggested. Even this is higher than the estimated 1.8% (83 spp.) of global species extinction in Earth's mammal fauna (even though the areas lost in species extinctions have not been estimated and included in population losses), about double the proportion of continental mammal species that have disappeared (less than 1%) (*5*).

Our estimates of population extinctions are necessarily crude. In addition, there are probably two major sources of conservative bias in our study, almost certainly leading to the substantial underestimation of those extinctions. First, even when the distribution of a charismatic endangered species is mapped, the existence of the species in some parts of its "present range" remains doubtful, as in the case of the tiger (*Panthera tigris*) [(*13*) and references therein; J. Ranganathan, personal communication]. We suspect that many less-prominent species, underrepresented in our sample, have lost portions of their ranges but without detection because they have not been subject to intensive mapping attempts.

The second probable conservative bias is potentially even greater. Distribution maps of historic ranges necessarily neglect the many smaller gaps in the distribution representing areas of unsuitable habitat (to take an obvious case, lakes and rivers do not ordinarily appear as blanks in the middle of prairie dog distributions). But we can be sure that anthropogenic habitat alteration has generally created much bigger gaps in the continuous maps that represent present distributions. For example, the map in the standard butterfly guide (*29*) shows the intensely studied *Euphydryas editha* as still occupying almost all of California except the Central Valley. In reality, population extinctions in historic times have removed it from many, if not most, of the sites where it occurred previously (*30*). Similarly, several species such as the monkeys *Leontopithecus rosalia* and *Brachyteles arachnoids* in the Mata Atlantica or the marsupials *Phascogale calura* and *Sminthopsis longicaudata* in Australia have had their historic ranges reduced to tiny fragments of habitat (*12*, *19*, *25*). Nonetheless, they are shown in our present maps as occupying entire quadrats, even though the vast majority of the populations in those quadrats have already gone extinct. If such smaller scale but nearly ubiquitous differences between historic and present mammal distributions could be calculated, losses of area and populations would be much greater.

There is a need to determine more precisely the proportion of mammal species that are shrinking on continents other than Australia, the one continent that has been relatively thoroughly studied, and to investigate the relation of vulnerability to population extinction with respect to body size and other variables on those continents. Also, studies of the details of "range filling" in mammals and other organisms will be critical to measuring more accurately the magnitude of population extinctions. An especially difficult problem is to translate between loss of range area and extinction of populations (*9*).

Table 1. Average area losses in mammals whose ranges have contracted. Samples were taken from six continents. Asterisk indicates value is from raw data, not from columns to the left.

Continent	No. of species	Historic range (km^2/1000)	Present range (km^2/1000)	Range lost (km^2/1000)	% Range lost*
Africa	52	5750	2046	3704	72
North America	18	4735	2761	1974	44
South America	17	5467	4648	819	15
Southeast Asia	13	2677	384	2293	83
Australia	58	1006	252	754	78
Europe	15	3628	1122	2506	72
Total	173				
Grand mean		3599	1569	2030	68

By definition, conserving population diversity means spreading conservation efforts over wider regions as a complement to important efforts to preserve "hotspots" of species richness (*31*, *32*). Such a regional approach will be made more difficult by the problem of what we call "political endemism," the limitation through population extinctions of a species' geographic range to one or a few political entities. In some cases, if such political entities are not as interested (or capable) in conservation as other entities in the historic range, that may ensure eventual extinction (*33*). A combination of political endemism and political instability has certainly made the fates of the black (*Diceros bicornis*) and Sumatran (*Dicerorhinus sumatrensis*) rhinos much more uncertain (*34*). In both of these conservation cases, a high priority would be to reestablish populations not only over a broader geographic range, but also within a greater variety of countries.

The loss of species diversity has correctly attracted much attention from the general public and decision-makers. It is now the job of the community of environmental scientists to give equal prominence to the issue of the loss of population diversity.

References and Notes

1. G. C. Daily, Ed., *Nature's Services: Societal Dependence on Natural Ecosystems* (Island Press, Washington, DC, 1997).
2. J. H. Brown, M. Lomolino, *Biogeography* (Sinauer, Sunderland, MA, 1998).
3. S. L. Pimm, J. L. Gittleman, T. M. Brooks, *Science* **269**, 347 (1995).
4. R. D. E. MacPhee, Ed., *Extinctions in Near Time: Causes, Context, and Consequences* (Kluwer Academic/Plenum, New York, NY, 1999).
5. C. Hilton-Taylor, *2000 IUCN Red List of Threatened Species* (IUCN, Gland, Switzerland, 2000).
6. M. V. Lomolino, R. Channel, *J. Mammal.* **76**, 335 (1995).
7. J. Short, *Biol. Conserv.* **86**, 365 (1998).
8. R. Channell, M. V. Lomolino, *J. Biogeogr.* **27**, 169 (2000).
9. J. B. Hughes, G. C. Daily, P. R. Ehrlich, *Science* **278**, 689 (1997).
10. J. L. Gittleman, M. E. Gompper, *Science* **291**, 997 (2001).
11. R. D. E. MacPhee, C. Flemming, *Science* **292**, 217 (2001).
12. M. Cardillo, L. Bromham, *Conserv. Biol.* **15**, 1435 (2001).
13. J. Seidensticker, S. Christie, P. Jackson, *Riding the Tiger: Tiger Conservation in Human Dominated Landscapes* (Cambridge Univ. Press, Cambridge, 1999).
14. R. K. Laidlaw, *Conserv. Biol.* **14**, 1639 (2000).
15. A. Balmford *et al.*, *Science* **291**, 2616 (2001).
16. J. Kingdon, *The Kingdon Field Guide to African Mammals* (Academic Press, London, 1997); J. L. Cloudsley-Thompson, *Environ. Conserv.* **1**, 5 (1974).
17. J. E. Fa, J. E. Garcia Yuste, R. Castelo, *Conserv. Biol.* **14**, 1602 (2000).
18. D. S. Wilkie, J. G. Sidle, G. C. Boundzanga, S. Blake, P. Auzel, in *Impacts of Commercial Logging on Wildlife in Tropical Forests*, R. Fimbel, A. Grajal, J. G. Robinson, Eds. (Yale Univ. Press, New Haven, CT, 2000), pp. 375–399.
19. J. Short, A. Smith, *J. Mammal.* **75**, 288 (1994).
20. D. B. Lindenmayer, H. P. Possingham, *The Risk of Extinction: Ranking Management Options for Lead-*

beater's Possum using Population Viability Analysis (Centre for Resource and Environmental Studies, Australian National University, Canberra, 1995).
21. M. D. Fox, D. Adamson, in *A Natural Legacy*, H. F. Recher, D. Lunney, I. Dunn, Eds. (Pergamon Press, Sydney, Australia, 1986), pp. 235–255.
22. J. A. Friend, *Proc. Ecol. Soc. Aust.* **16**, 369 (1990).
23. C. Bailey, *Landscope* **11**, 41 (1996).
24. J. E. Kinnear, M. L. Onus, N. R. Sumner, *Wildl. Res.* **25**, 81 (1998).
25. G. B. A. Da Fonseca, *Biol. Conserv.* **34**, 17 (1985).
26. V. G. Roig, in *Latin American Mammalogy: History, Biodiversity, and Conservation*, M. A. Mares, D. J. Schmidly, Eds. (Univ. Oklahoma Press, Norman, OK, 1991), pp. 239–279.
27. G. B. Corbet, H. N. Southern, Eds., *The Handbook of British Mammals* (Blackwell Scientific, London, 1977).
28. A. J. Mitchell-Jones *et al.*, *The Atlas of European Mammals* (Academic Press, London, 1999).
29. J. A. Scott, *The Butterflies of North America: A Natural History and Field Guide* (Stanford University Press, Stanford, CA, 1986).
30. D. D. Murphy, S. B. Weiss, *Biol. Conserv.* **46**, 183 (1988).
31. R. A. Mittermeier, N. Myers, P. R. Gil, C. G. Mittermeier, *Hot Spots: Earth's Biologically Richest and Most Endangered Terrestrial Ecosystems* (CEMEX and Conservation International, Monterrey, Mexico, 1999).
32. N. Myers, R. A. Mittermeier, C. G. Mittermeier, G. A. B. da Fonseca, J. Kent, *Nature* **403**, 853 (2000).
33. G. Ceballos, J. H. Brown, *Conserv. Biol.* **9**, 559 (1995).
34. C. Santiapillai, K. MacKinnon, in *Rhinoceros Biology and Conservation*, O. A. Ryder, Ed. (San Diego Zoological Society, San Diego, CA, 1993), pp. 257–264.

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Supporting Online Material
(www.sciencemag.org/cgi/content/full/296/5569/904/DC1) table S1
References and notes

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Genomewide Analysis of mRNA Processing in Yeast Using Splicing-Specific Microarrays

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Introns interrupt almost every eukaryotic protein-coding gene, yet how the splicing apparatus interprets the genome during messenger RNA (mRNA) synthesis is poorly understood. We designed microarrays to distinguish spliced from unspliced RNA for each intron-containing yeast gene and measured genomewide effects on splicing caused by loss of 18 different mRNA processing factors. After accommodating changes in transcription and decay by using gene-specific indexes, functional relationships between mRNA processing factors can be identified through their common effects on spliced and unspliced RNA. Groups of genes with different dependencies on mRNA processing factors are also apparent. Quantitative polymerase chain reactions confirm the array-based finding that Prp17p and Prp18p are not dispensable for removal of introns with short branchpoint-to-3′ splice site distances.

Protein-coding information in eukaryotic genomes is fragmented into exons, which must be recognized and joined by the process of RNA splicing. Splicing takes place in the nucleus within a dynamic ribonucleoprotein complex called the spliceosome (*1*). The spliceosome transforms information within transcripts of the eukaryotic genome to create sequences not found in DNA. By its nature and position in the gene expression pathway, splicing expands the possible interpretations of genomic information and does so under developmental and environmental influence (*2*). Our understanding of the process of splicing is derived from studies on relatively few introns. As eukaryotic genomes are sequenced, it has become necessary to ask how the process of splicing is integrated into genome function and evolution. Compared with higher eukaryotes, yeast contains relatively few spliceosomal introns, and most have been correctly annotated (*3*, *4*). Hence, we chose to perform genomewide study of splicing in the yeast *Saccharomyces cerevisiae*.

To discriminate between spliced and unspliced RNAs for each intron-containing yeast gene, we used DNA microarrays (*5*, *6*). Oligonucleotides were designed to detect the splice junction (specific to spliced RNA and not found in the genome), the intron (present in unspliced RNA), and the second exon (common to spliced and unspliced RNA) for each intron-containing gene as shown in Figure 1A. The oligonucleotides were printed on glass slides to create splicing-sensitive microarrays for yeast (*7*).

To determine whether oligonucleotide arrays can function as genomewide sensors of splicing, we compared RNA of cells carrying the temperature-sensitive splicing mutation *prp4-1* with RNA of wild type during a shift from 26°C to 37°C (*7*). Prp4p is an integral component of the spliceosome (*8*, *9*). Plots of fluorescence (*10*) for each oligonucleotide for the wild-type (Cy3) versus the *prp4-1* mutant (Cy5) with time are shown in Fig. 1B. Even at the permissive temperature of 26°C, many intron probes (red spots) display Cy5/Cy3 ratios >1, indicating accumulation of intron-containing RNA in the mutant strain. After the shift to the restrictive temperature, the Cy5/Cy3 ratio increases for most intron probes. In contrast, the ratio decreases for many splice junction probes (green spots), a sign that spliced RNAs become depleted in the mutant. The Cy5/Cy3 ratios for about a thousand intronless genes remain largely unaffected (yellow spots). This indicates that the array reports catastrophic splicing defects and can measure the kinetics of splicing inhibition genomewide.

Despite their conservation, numerous mRNA processing factors are not essential in yeast. To analyze more subtle changes in splicing, we studied 18 mutant strains lacking nonessential genes implicated in mRNA processing (Table 1). Plots of mutant versus wild-type fluorescence intensities for *prp18*Δ, *cus2*Δ, and *dbr1*Δ are shown in Fig. 1C. The effect of each deletion on spliced and unspliced RNA is different. Most severe is *prp18*Δ, which causes widespread intron accumulation and loss of splice junction sequences relative to wild type (Fig. 1C, left). The *cus2*Δ mutation enhances defects in U2 small nuclear RNA (snRNA) or Prp5p (*11*, *12*) but causes little intron accumulation (Fig. 1C, center). Although not required for splicing, Dbr1p debranches the lariat, and its loss results in the dramatic accumulation of intron lariats (*13*). In the *dbr1*Δ strain, most introns accumulate, and there is little effect on spliced mRNAs (Fig. 1C, right). This demonstrates that qualitative differences in splicing phenotype can be distinguished by using splicing sensitive microarrays.

Changes in spliced and unspliced RNA levels due to loss of an mRNA processing factor may arise directly from splicing inhibition or may be due to secondary events that alter transcription or RNA decay. For example, signal from a splice junction probe may increase for a gene whose transcription is induced, even though splicing is inhibited. To

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